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Evaluation of Selected Commercial Oils as Oviposition Deterrents Against the Silverleaf Whitefly, *Bemisia argentifolii* (Hemiptera: Aleyrodidae)

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ABSTRACT

Silverleaf whitefly (SLWF), *Bemisia argentifolii* Bellows and Perring, remains a serious economic pest of vegetables and ornamentals worldwide. Conventional chemical control of whiteflies is often rendered ineffective due to rapid development of insecticide resistance. However, relatively little research effort has been reported on cultural management techniques, such as habitat manipulations that could mitigate economic loss due to whitefly damage. The use of extracts and oils of certain other plants as repellents or oviposition deterrents may be an important cultural management technique. We evaluated four commercial oils as repellents or oviposition deterrents against the silverleaf whitefly ("push" factor) as a component of the "push-pull" management strategy. The "push-pull" strategy involves the behavioral manipulation of insect pests and natural enemies through stimuli that make the target crop unattractive to the pest ("push" strategy), while simultaneously attracting them to a trap crop ("pull" strategy) where they are removed, preferably through biological control or other appropriate means. Potted squash plants (*Cucumis moschata* Duchesne ex Poir) were sprayed with the following four oil treatments at recommended label rates: garlic oil (11%), hot pepper wax oil (3%), mustard oil (3%) and horticultural petroleum oil (1%). Tween 20® (2%) was used as a spreader in all solutions. Water + Tween 20® solution served as the control. SLWF adults were released in no-choice and choice experiments for 24 hrs. In the no-choice experiment, we found significantly higher numbers of SLWF eggs in the control and hot pepper wax oil treatments compared to those sprayed with treatments of garlic oil, mustard oil and horticultural petroleum oil. In the choice experiment, mustard oil significantly reduced both numbers of SLWF adults and newly-laid eggs. The results for the horticultural petroleum oil treatment were not as clear. Although SLWF adult counts were significantly reduced, egg counts were significantly reduced only at the 10 % level ($P = 0.054$). These results indicate that among the evaluated oils, the mustard oil treatment is most promising as the "push" component in a "push-pull" management program. This study will be useful in enhancing integrated pest management strategies against the silverleaf whitefly pest in vegetable and horticultural crops.

Additional Index Words: mustard oil, plant repellents, vegetable crops, push-pull strategies.

Despite significant research efforts since the 1990s, the silverleaf whitefly (SLWF), *Bemisia argentifolii* (Bellows and Perring) (= *B. tabaci* (Gennadius) Biotype B) (Hemiptera: Aleyrodidae), remains a serious economic pest of vegetables and ornamentals worldwide. Conventional chemical control of this whitefly is often rendered ineffective due to rapid development of insecticide resistance (Palumbo et al. 2001). However, relatively little research effort has been reported on cultural management techniques, such as habitat manipulations that could mitigate economic loss due to whitefly damage (Naranjo 2001, Potting et al. 2005). An important cultural management technique may be the use of extracts and oils of other plants as repellents or oviposition deterrents (Zhang et al. 2004, Al-mazra'awi and Ateyyat 2009). Although host location is largely dependent on visual cues (Mound 1962), olfactory cues are also important (Bleeker et al. 2009) and whiteflies are capable of direct, active flight (Byrne 1999). Recent olfactometer experiments demonstrated possible repellent effects of mustard

(*Brassica juncea* (L.) Czern) volatiles to whiteflies (Legaspi et al. 2011). Planting mustard as a companion crop with collards (*Brassica oleracea* L. var. *acephala*) in outdoor experiments resulted in reduced whitefly landings, but not in reduced oviposition on the collards (Legaspi et al. 2011).

The study described herein is a component of a more comprehensive research effort to develop a "push-pull" strategy for management of the silverleaf whitefly. The "push-pull" strategy involves the behavioral manipulation of insect pests and natural enemies through stimuli that make the target crop unattractive to the pest ("push" strategy), while simultaneously attracting them to a trap crop ("pull" strategy) where they are removed, preferably through biological control (Cook et al. 2007) or other appropriate means (Shelton and Badenes-Perez 2006). Herein, we evaluated selected commercial oils as repellents or oviposition deterrents against the whitefly ("push" factor) as a component of the "push-pull" strategy.

MATERIALS AND METHODS

To quantify the effects of commercially available repellent products on oviposition by *B. argentifolii*, we applied four commercial oil products to squash plants (*Cucurbita moschata* Duchesne ex Poir., var. "Dixie Hybrid") at the label recommended rates in two experimental settings and locations. The no-choice experiment was conducted at USDA-ARS in Tallahassee, FL and the choice experiment was conducted at USDA-ARS in Charleston, SC. The commercial oil treatments tested in both the no-choice and choice experiments included: Garlic Barrier®applied at a rate of 11% (Garlic Research Labs Inc., Glendale, CA), Hot Pepper Wax® applied at a rate of 3% (Hot Pepper Wax Inc., Greenville, PA), Organic Mustard Seed Oil® applied at a rate of 3% (Botanic Oil Innovations, Spooner, WI), and the horticultural petroleum Year-Round Spray Oil® at a rate of 1% (Summit Responsible Solutions, Baltimore, MD). These products will hereafter be referred to as garlic oil, hot pepper wax, mustard oil and horticultural petroleum oil, respectively. Tween 20® (Cayman Chemical Co., Ann Arbor, MI) was used as a spreader in all treatment solutions at a rate of 2%. Water + Tween 20® solution served as the control.

No-Choice experiment. A colony of *B. argentifolii*, has been maintained since 2008 at the USDA-ARS-CMAVE Center for Biological Control in Tallahassee, FL on a mixed regime of potted collards and tomato. This colony was used for the no-choice experiment. Potted squash plants (15.2 cm diam) with about 6 leaves were sprayed 1.5 h prior to testing with hand held plastic sprayers. The plants were allowed to dry completely before they were placed into round plastic containers (36 cm height x 11 cm diam.) with screened lids. A cut microcentrifuge tube was placed in the side of each container to serve as an opening to release whitefly adults. Twenty adult SLWF females were placed into each potted container. The potted containers were placed in ThermoForma Model 3740 growth chambers (ThermoForma, Marietta, OH) at 25° C and a 14:10 L:D photoperiod. Six to seven plants per oil treatment were placed in a separate growth chamber to avoid mixing of volatiles in the enclosed space. The experiment was repeated at three different times with chambers randomly assigned to each oil treatment. There were a total of 19 replicates per treatment. After 24 h, the plants were removed from the chambers and the leaves were removed. Eggs were counted on each leaf surface and the data were recorded.

Choice experiment. A separate colony of *B. argentifolii* was maintained at the USDA-ARS, U.S. Vegetable Laboratory in Charleston, SC. Insects from this colony were reared on several vegetables in the greenhouse according to Simmons 1994. Potted squash plants (var. "Dixie hybrid") with about 6 leaves were sprayed with a hand sprayer using the same commercial oils and recommended label rates as the no-choice experiment above. Water + Tween 20® solution served as the control. The potted plants were left to dry completely after spraying. Two potted plants (treated and untreated control) (15.2 cm diam.) were placed in opposite corners of a screened cage (61 cm height x 61 cm width x 61 cm depth, BioQuip Products, Inc., Rancho Dominguez, CA). In each trial, there were five potted plants per treatment paired with a control. The experiment was repeated five different times (i.e., trials) for a total of 25 replicates per treatment and control. The screened cages were kept outdoors throughout the experiment. Temperature and humidity were monitored and recorded using an HOBO® recorder (Onset Computer Corp., Bourne, MA). For each cage, two hundred unsexed adult SLWF from the colony were aspirated into a blackened collecting vial. The vial of whiteflies was covered with a lid that had a 4 mm opening and it was placed in the middle of the cage to release the whiteflies. After 24 hr, the numbers of adults on each leaf per plant were counted before collecting the leaves. The leaves were returned to the laboratory for observation with the aid of a microscope. The number of newly-laid SLWF eggs on each leaf surface were counted and recorded.

Statistical analysis. In the no-choice experiment, numbers of eggs laid were analyzed by 1-way ANOVA, and means were separated using

Tukey's HSD ($P < 0.05$). In the choice experiment, statistical analysis was performed separately on each commercial oil or control using paired t-tests to compare numbers of whitefly counts or eggs between treated plants and the corresponding control plant after 24-h exposure to the whiteflies. All analyses were performed using Systat 12 (Systat Software Inc., Chicago, IL).

RESULTS

No-Choice experiment. Significantly different numbers of eggs were laid on leaves in the different treatments ($F = 4.96$; df = 4, 87; $P = 0.001$; $R^2 = 0.43$) (Fig. 1). Highest numbers of eggs were found in the control and hot pepper wax oil treatments. Fewer eggs were found in the garlic oil, mustard oil and horticultural petroleum oil treatments (Tukey's HSD; $P < 0.05$). Numbers of eggs per leaf averaged 15 to 20 in the horticultural petroleum oil, mustard oil and garlic oil treatments, compared to about 55 in the control.

Choice experiment. During the experiment, the minimum and maximum relative humidity was 38% and 98%, respectively. The minimum and maximum temperature was 19 ^{o}C and 34 ^{o}C, respectively. Results of the choice experiment are summarized in Table 1. Mustard oil was found to significantly reduce both numbers of adult whitefly counts and numbers of SLWF eggs counted. The results for the horticultural petroleum oil treatment were not as clear in this experiment. Although SLWF adult counts were significantly reduced, egg counts were significantly reduced only at the 10% level ($P = 0.054$).

DISCUSSION

Olfactometer experiments using entire mustard plants showed evidence of repellency to whitefly adult females and reduced landings in collards intercropped with mustard (Legaspi et al. 2011). However, the reduced landings did not translate into reduced oviposition on the collards (Legaspi et al. 2011). In another study testing collard and mustard plants in the same pots in the greenhouse, the proximity of the mustard seemed to reduce eggs counts on the adjacent collard plant, and field counts of whitefly eggs were lower on mustard compared to collards and other field crops in the field (Legaspi 2010). However, the presence of mustard crop transects did not appear to reduce whitefly oviposition in nearby crops. These companion studies suggest that volatiles from whole mustard plants only have a weak repellent effect on the whiteflies which often does not result in measurable reduction in oviposition on an economic crop, thereby indicating the need to evaluate commercial extracts.

In a related study using no-choice and choice experiments, kaolin-based particle film and a mineral oil did not reduce *B. argentifolii* oviposition alone or in combination when compared to a water control (Liang and Liu 2002). However, in the choice experiments, oviposition was lower in the treated leaves compared to the water control (Liang and Liu 2002). Ginger oil was evaluated for repellency to *B. argentifolii* using a vertical still-air olfactometer, as well as choice and no-choice experiments (Zhang et al. 2004). Ginger oil odor was found effective at distances of 1 – 2 mm. In a greenhouse choice test, 35 – 42% fewer whitefly adults settled and 37% fewer eggs were laid during 24 h on tomato seedlings dipped in 0.25% ginger oil + 2% Tween®, compared to seedlings dipped in the 2% Tween® control. In the no-choice test, 10.2 – 16.7% fewer whiteflies settled compared to the control, but there were no significant differences in the numbers of eggs laid (Zhang et al. 2004). Effectiveness of ginger oil as a repellent was mitigated by issues related to inadequate coverage and phytotoxicity.

In our study, we found that the repellent oil treatments generally reduced numbers of eggs deposited by the whitefly, although the reduction was modest and will likely require complementary controls for effective whitefly management. The effect on oviposition was because of the impact that certain repellents had on the adults. This work demonstrates that some spray treatments can have a greater impact on the behavior of whiteflies than others. This study did not discern between whether adult whitefly landed on leaves and left because of taste or odor versus whiteflies who may have not reached the leaves because of the odor. Further olfactometer and cage studies on SLWF adult behavior can clarify these observations. Future studies can focus on the mustard oil as the "push" component in a "push-pull" management program.

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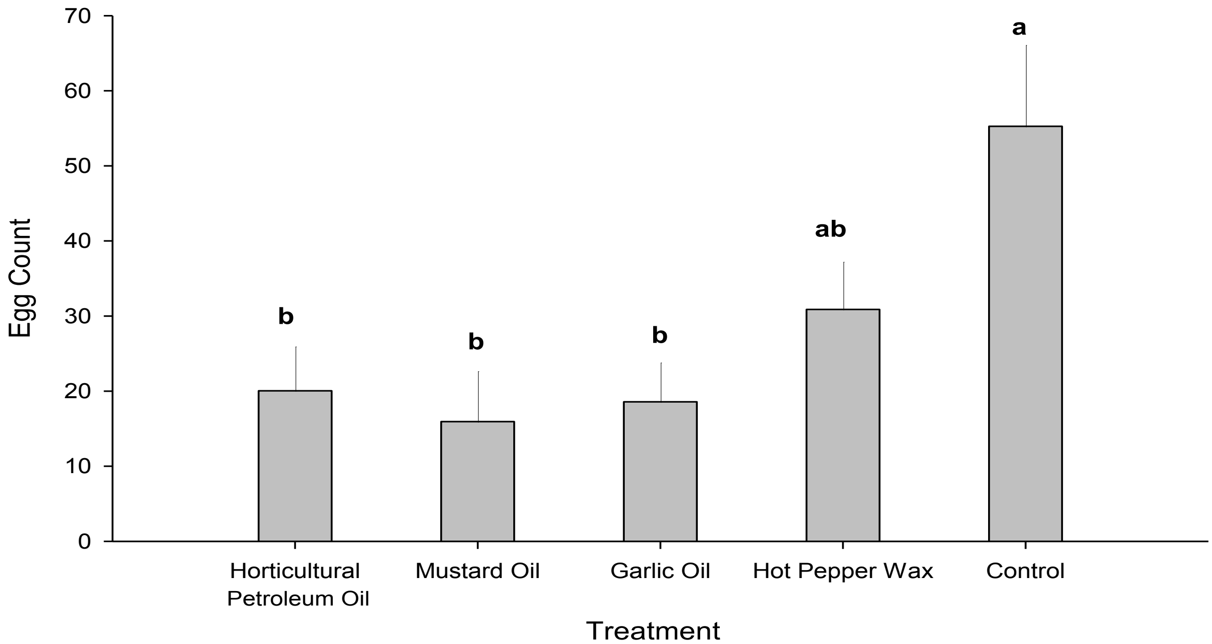

Fig. 1. <u>No-choice experiment.</u> Mean numbers (± SE) of *B. argentifolii* eggs laid on squash plants sprayed with four different commercial oil oviposition repellents and a control after 24 h exposure. Different letters indicate that means were significantly different (Tukey HSD, $P < 0.05$)

Table 1. <u>Choice experiment.</u> Adult whitefly counts and numbers of eggs laid (mean ± SE) for treated and control plants 24 h after exposure to silverleaf whiteflies. Tests compared numbers between commercial oil treated plants and corresponding control plants in the same replicate. For each treatment, paired t-tests were used to compare numbers between treated and corresponding control plants.

Treatment	Whitefly counts		Egg counts	
	Treated	Control	Treated	Control
Mustard Oil	23.2 ± 2.9	46.2 ± 6.1	98.8 ± 20.8	187.6 ± 35.8
	(t = -3.66; df = 24; *P* < 0.01)		(t = -2.5; df = 24; *P* = 0.019)	
Garlic Oil	40.3 ± 6.2	51.4 ± 8.0	157.2 ± 26.1	213.9 ± 33.8
	(t = -1.22; df = 24; *P* = 0.234)		(t = -1.68; df = 24; *P* = 0.105)	
Hot Pepper Wax	32.9 ± 6.1	42.9 ± 7.9	120.6 ± 20.0	172.0 ± 29.2
	(t = -0.97; df = 24; *P* = 0.342)		(t = -1.42; df = 24; *P* = 0.168)	
Horticultural Petroleum Oil	28.5 ± 5.5	49.5 ± 7.7	116.6 ± 30.6	195.4 ± 33.8
	(t = -2.19; df = 24; *P* = 0.038)		(t = -2.022; df = 24; *P* = 0.054)	

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